PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 38/22, 38/07 — A2

(11) International Publication Number: **WO 99/65513**

(43) International Publication Date: 23 December 1999 (23.12.99)

(21) International Application Number: PCT/IL99/00335

(22) International Filing Date: 17 June 1999 (17.06.99)

(30) Priority Data:

60/089,770	18 June 1998 (18.06.98)	US
60/147,195	20 May 1999 (20.05.99)	US

(71)(72) Applicants and Inventors: CHOWERS, Michal, Y. [IL/IL]; P.O. Box 77, 44925 Tzufit (IL). CHOWERS, Yehuda [IL/IL]; P.O. Box 77, 44925 Tzufit (IL).

(74) Agents: LUZZATTO, Kfir et al.; Luzzatto & Luzzatto, P.O. Box 5352, 84152 Beer-Sheva (IL).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF *HELICOBACTER PYLORI*-ASSOCIATED DISORDERS

(57) Abstract

The invention relates to pharmaceutical compositions and methods for treating and/or preventing *Helicobacter pylori*-associated disorders, particularly disorders of the gastrointestinal tract. The pharmaceutical compositions comprise as active ingredient a therapeutically effective amount of a compound which inhibits the growth-enhancing effect of gastrin on *H. pylori*. The active ingredient may specifically be a compound which is capable of inhibiting gastrin uptake by *H. pylori*, and/or which is an antagonist of the human or *H. pylori* gastrin receptor.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

Pharmaceutical Compositions for the Treatment of *Helicobacter pylori*-associated Disorders

Field of the Invention

The invention relates to pharmaceutical compositions for the treatment and/or prevention of *Helicobacter pylori*-associated disorders and to methods of treating such disorders in patients in need thereof.

Background of the Invention

Helicobacter pylori (*H. pylori*) infection is associated with several benign and malignant human diseases [Dooley CP, *et al.*, N Engl J Med (1989) **321**:1562-1566; Carrick J, *et al.*, Gut (1989) **30**:790-797; Nomura A, *et al.*, N Engl J Med (1991) **325**:1132-1136 [see comments]; Nomura A, *et al.*, Ann Intern Med (1994) **120**:977-981 [see comments]; Zucca E, *et al.*, N Engl J Med (1998) **338**:804-810]. However, symptomatic infection is only the tip of the iceberg, the majority of infected individuals remain asymptomatic. Moreover, if untreated, infection may last for decades [Peterson WL and Harford WV, (1991) **86**:671-675], representing a successful host-parasite relationship. This favorable interaction is reflected in a high prevalence of infection ranging between 50 % in developed countries to 90 % in developing countries [Graham DY, *et al.*, Dig Dis Sci (1991) **36**:1084-1088; Taylor DN and Blaser MJ, Epidemiol Rev (1991) **13**:42-59].

H. pylori resides within the mucous layer of the human gastric mucosa. Due to extremely low pH, the stomach is a hostile environment to most other microorganisms. The ability of *H. pylori* to flourish in the stomach has been attributed to protective mechanisms such as its production of urease, protecting the bacterium from gastric acidity by creating a basic microenvironment

[Taylor DN and Blaser MJ, Epidemiol Rev (1991) **13**:42-59]. However, it has now been reasoned, that *H. pylori* might have evolved a way to gain growth advantage in this particular niche of the stomach, possibly by exploiting a gastric factor. A logical candidate would be one that is upregulated by *H. pylori* infection.

One such factor is the gastric hormone gastrin. Gastrin is produced as a prohormone by G cells located within the gastric antrum. The prohormone is later processed to shorter peptides, the most abundant of which is 17 amino acids long, termed gastrin 17 (G17) [Eaton KA, *et al.*, Infect Immun (1991) **59**:2470-2475]. The major role attributed to gastrin within gastric tissue is the regulation of acid secretion. Following infection, gastrin levels are found to be consistently elevated and normal physiologic negative feedback control of secretion is lost. Further, following *H. pylori* eradication, gastrin levels are reduced and normal feedback control of gastrin secretion is restored [Graham DY, *et al.*, Am J Gastroenterol (1990) **85**:394-398; El-Omar E, *et al.*, Gut (1993) **34**:1060-1065 [see comments]; Konturek JW, *et al.*, Gut (1995) **37**:482-487].

Interest in the changes of gastrin secretion and control have been directed to its role in acid production, and the resulting peptic pathology. However, the present work was focused on the possible interactions between gastrin and *H. pylori.*

As will be shown hereafter, gastrin positively stimulates the growth of *H. pylori*, a finding which further exemplifies the successful adaptation of *H. pylori* to the human host, and provides the basis for the present invention.

Summary of the Invention

The present invention relates to pharmaceutical compositions for the treatment and/or prevention of *H. pylori*-associated disorders comprising as

active ingredient a therapeutically effective amount of a compound which inhibits the growth-enhancing effect of gastrin on *H. pylori*, particularly *H. pylori*- associated gastrointestinal disorders, such as *H. pylori*-associated gastric and/or duodenal peptic diseases. The pharmaceutical compositions of the invention may optionally further comprise pharmaceutically acceptable carriers, adjuvants or diluents.

The active ingredient in the pharmaceutical compositions of the invention may be a compound which is capable of inhibiting gastrin uptake by *H. pylori*, particularly compounds which are competitive inhibitors of gastrin uptake by *H. pylori*, or antagonists of the human or *H. pylori* gastrin receptor. In preferred embodiments, the active compound in the pharmaceutical compositions of the invention is a peptide. Preferred peptides are synthetic analogues of gastrin or of a fragment of gastrin, preferably of G17, and most preferred are peptides comprising the amino acid sequence: Trp-Met-Asp-PheNH_2, such as pentagastrin or cholecystokinin (CCK)-8.

In other embodiments the pharmaceutical compositions of the invention may comprise as the active ingredient a non-peptidic antagonist of the human or *H. pylori* gastrin receptor.

In yet a further aspect, the invention relates to a method for the treatment and/or prevention of *H. pylori*-associated disorders in a patient in need of such treatment, comprising administering to said patient a therapeutically effective amount of a compound which inhibits the growth-enhancing effect of gastrin on *H. pylori* or a therapeutically effective amount of a composition according to the invention.

The method of the invention may be used for the treatment and/or prevention of *H. pylori*-associated gastrointestinal disorders.

Yet further, the invention relates to use of a compound which inhibits the growth-enhancing effect of gastrin on *H. pylori* in the preparation of a pharmaceutical composition for the treatment of *H. pylori*-associated disorders. In the use according to the invention, the said compound may be a synthetic analogue of G17, preferably comprising the amino acid sequence: Trp-Met-Asp-PheNH_2. Alternatively, the compound may be a non-peptidic antagonist of the human or *H. pylori* gastrin receptor.

Brief Description of the Figures

Figures 1A to 1B *H. pylori growth kinetics in the presence of gastrin.*

Bacteria were grown in liquid media, using microaerophilic conditions with increasing gastrin concentrations. Growth rate was assessed by optical density (OD). T indicates time in hours.

Figure 1(A) shows a representative growth curve of one isolate over 48 hours.

Figure 1(B) shows cumulated growth data of 5 different clinical isolates with (3.7 pM) or without gastrin. Growth is compared at 0h and 48h (Shown as a mean with SEM).

Figures 2A to 2F *Gastrin growth stimulation is bacterial and peptide specific.*

Growth kinetic analysis of different bacteria with gastrin:

Figure 2A: Growth kinetics analysis of *C. jejuni* with gastrin;

Figure 2B: : Growth kinetics analysis of *E. coli* with gastrin;

Figure 2C: Growth kinetics analysis of *H pylori* with the gastric peptide somatostatin;

Figure 2D: Growth kinetics analysis of *H pylori* with EGF.

Figure 2E: Growth kinetics analysis of *H. pylori* with human gastrin receptor agonist pentagastrin;

Figure 2F: Growth kinetics analysis of *H. pylori* with human gastrin receptor agonist CCK-8.

Bacteria were grown using liquid media and growth rate assessed by optical density (OD). T indicates time in hours. Except the aerobic conditions used for the growth of *E. coli*, all growth conditions were identical. One experiment representative of three (each performed with a different isolate).

Figure 3 *Gastrin uptake assay.*

H. pylori (H.P.), *E. coli* (E.C.), *S. pneumonia* (S.P.) and *C. jejuni* (C.J.) bacteria were incubated for 45 minutes with ^{125}I-labeled gastrin. Incubation was at 4^{0}C or 37^{0}C. Following initial incubation, proteinase K (P.K) was added for 30 minutes, after which bacteria were washed and blotted. One experiment representative of two.

Figure 4 *Competition by different peptides on ^{125}I-labeled gastrin uptake by H. pylori.*

Bacteria were incubated in the presence of 500 nmol/l ^{125}I-labeled gastrin and increasing concentrations of either unlabeled gastrin (G), CCK-8 (CCK), pentagastrin (Pen), somatostatin (Som) or EGF for 45min. Bacteria were washed and radioactivity remaining in bacterial pellets was determined. One experiment representative of three.

Detailed Description of the Invention

H. pylori infection is associated with duodenal ulcer, gastric ulcer, gastric adenocarcinoma and B cell lymphoma. It has also been implicated in cardiovascular diseases, particularly atherosclerosis and ischemia, since it has

been shown that the infection is more abundant in patients suffering from these diseases. The bacterium resides within the gastric mucosa, a unique niche hostile to other microorganisms. Gastrin, a gastric hormone, is consistently elevated following *H. pylori* infection. It has now been found that human gastrin stimulates *H. pylori* growth in a specific, dose-dependent mechanism, which suggests a novel approach to treating *H. pylori* infections.

As demonstrated in the following Examples, the human hormone gastrin significantly stimulated *H. pylori* growth in a dose-dependent manner. This effect was reproducible in all bacterial isolates studied. Growth stimulation occurred at physiological gastrin concentrations found in the blood and gastric lumen [Mueller CR, *et al.*, Surgery (1991) **110**:1116-1124; Yamashita K, *et al.*, Gastroenterology (1998) **115**:1123-1130].

The uptake of gastrin by *H. pylori* and the stimulatory growth effect were highly specific and were not detected with control bacteria or by other peptides present in the gastric antrum or lumen. Moreover, cold ligand inhibition suggested the effect was mediated via a specific gastrin binding site. Gastrin, CCK-8 and pentagastrin have structural similarity and activate human gastrin/CCKB receptors. In the present experiments, both CCK-8 and pentagastrin competed with gastrin on the binding to *H. pylori*, but did not stimulate bacterial growth. The competition by structurally related compounds supports the suggestion that a specific binding site is involved in gastrin uptake by *H. pylori*. The fact that biological activity could be obtained only with gastrin, reiterates the specific interaction of *H. pylori* with this hormone.

In light of the present findings, host parasite interaction between the human host and *H. pylori* could be viewed as having evolved to exploit gastrin by the bacterium. *H. pylori* preferentially colonizes the gastric antrum, the anatomic location of gastrin producing G cells [Bayerdorffer E, *et al.*, Gastroenterology (1992) **102**:1575-1582]. In addition, infection results in

hypergastrinemia and a loss of negative feedback control on gastrin secretion, potentially leading to the continuous exposure of bacteria to high levels of gastrin.

The histologic hallmark of *H. pylori* infection is chronic antral inflammation. As a result of this inflammation, increased levels of pro-inflammatory cytokines have been documented [Crabtree JE, *et al.*, Gut (1991) **32**:1473-1477; Karttunen R, *et al.*, Gut (1995) **36**:341-345; Noach LA, *et al.*, Scand J Gastroenterol (1994) **29**:425-429; Crabtree JE, *et al.*, Scand J Immunol (1993) **37**:65-70]. Using *in vitro* cultures, it was shown that endocrine cells can produce gastrin in response to stimulation with such inflammatory cytokines [Lehmann FS, *et al.*, Am J Physiol (1996) **270**:783-788; Weigert N, *et al.*, Gastroenterology (1996) **110**:147-154; Beales IL, *et al.*, Eur J Gastroenterol Hepatol (1997) **9**:773-777; Wallace JL, *et al.* Am J Physiol (1991) **261**:G559-564]. Thus, *H. pylori* may actually benefit from the host immune response, which initiates up-regulation of a bacterial growth factor.

Models based on *H. pylori* characteristics and host parasite interaction have been proposed. Lee suggested that the exceptionally small size of the *H. pylori* genome and the small number of regulatory genes might indicate that the bacterium is well adapted to a highly specific single habitat [Lee A, N Engl J Med (1998) **338**:832-833]. The finding that *H. pylori* utilizes gastrin, a gastric hormone, concurs with this proposal. Furthermore, the chronicity and persistence of *H. pylori* infection in the human host led Blaser to suggest that the interaction between bacterium and host is mutually regulated. Such regulation implies co-evolution [Blaser MJ, J Clin Invest (1997) **100**:759-762]. Increased gastrin secretion as a consequence of infection and the possible exploitation of gastrin by the bacterium fit this concept.

Therapy of peptic ulcer disease prior to the discovery of the central role of H. pylori, was based on agents capable of reducing gastric acidity. Based on meta analysis of multiple trails aimed at healing peptic ulcers, it was shown that there is no advantage in elevating the pH to above 3, for more than 18 hours daily. Still, several groups of agents are used for reducing the stomach acidity. Most important of these agents are the H2 receptor antagonists and proton pump inhibitors. H2 receptor antagonists act by blocking histamine H2 receptors on the parietal cells. Proton pump inhibitors act by inhibition of the parietal cell H^+K^+ ATPase, responsible for acid secretion from the cells.

Current therapies of *H. pylori* infections usually consist of combinations of two antibiotic agents together with an adjunctive agent, which is usually either a proton pump inhibitor or bismuth. Antibiotic resistance of *H. pylori* is widespread, with increasing prevalence [Hazell, SL, Eur J Clin Infect Dis (1999) **18**:83-86]. H. pylori-associated infection is a rather common phenomenon, resulting in a massive use of antibiotics, sometimes prescribed also to a symptomatic carriers. This massive use may exhaust the option of treatment of this disease by currently available drugs, and the resistance of the bacterium may later adversely affect the extent of success of therapy of *H. pylori* infection, as well as other infectious agents that may become resistant, as a secondary effect of extensive use of antibiotics.

The present findings suggest that gastrin may be the factor on which *H. pylori* thrives, and in its absence, the bacterium will not be able to survive in the gastric antrum. The growth benefit provided to *H. pylori* by gastrin, may be the reason for the ability of this bacterium to flourish in this hostile habitat.

It is this newly discovered relationship between gastrin and *H. pylori*, which provides the basis for alternative, non-antibiotic-based improved therapies of various diseases associated with *H. pylori* infection, particularly those of the gastrointestinal tract.

Thus, the invention relates to a pharmaceutical composition for the treatment and/or prevention of *H. pylori*-associated disorders comprising as active ingredient a therapeutically effective amount of a compound which inhibits the growth-enhancing effect of gastrin on *H. pylori*, optionally further comprising pharmaceutically acceptable carriers, adjuvants or diluents.

The pharmaceutical composition of the invention may be particularly suitable for the treatment and/or prevention of *H. pylori*-associated gastrointestinal disorders, specifically, *H. pylori*-associated gastric and/or duodenal peptic diseases. In addition the compositions of the invention may be used for the treatment of gastritis, duodenitis, non-ulcer dyspepsia, mucosal-associated lymphoid tissue lymphoma, for the prevention of gastric carcinoma and in the treatment of atherosclerotic cardiovascular diseases.

The active ingredient in the pharmaceutical compositions of the invention is preferably a compound which is capable of inhibiting gastrin uptake by *H. pylori*. Such compound may be a competitive inhibitor of gastrin uptake by *H. pylori*, or an antagonist of the human or *H. pylori* gastrin receptor.

In preferred embodiments, the active compound comprised in the pharmaceutical composition of the invention is a peptide. Preferred peptides may be synthetic analogue of gastrin or of a fragment of gastrin, preferably comprising an amino acid sequence corresponding to the C-terminal of gastrin or to a gastrin fragment, preferably G17. Such preferred peptides comprise the amino acid sequence: Trp-Met-Asp-PheNH_2. Particular examples of synthetic peptides which may be used as the active principal in the compositions of the invention are pentagastrin or cholecystokinin (CCK)-8.

Pentagastrin is a synthetic gastrin analogue that exhibits the biological activity of gastrin. The main clinical use of this compound was for stimulation of acid secretion in a diagnostic test required for exclusion of achlorhydria

(acid stimulation occurs within 10 minutes and peaks within 20-30 minutes). Pentagastrin replaced the use of histamine in such tests, because it has milder side effects.

Synthetic peptides used in the compositions of the invention may be in the form of a dimer, a multimer or in a constrained conformation, in which the constrained conformation is obtained by internal bridges, short-range cyclizations, extension or other chemical modification.

In a further embodiment, the active compound comprised in the pharmaceutical compositions of the invention may be a non-peptidic antagonist of the human or *H. pylori* gastrin receptor.

In a further aspect, the present invention relates to a method for the treatment and/or prevention of *H. pylori*-associated disorders in a patient in need of such treatment, comprising administering to said patient a therapeutically effective amount of a compound which inhibits the growth-enhancing effect of gastrin on *H. pylori* or a therapeutically effective amount of a composition according to the invention.

The method of the invention is particularly suitable for the treatment and/or prevention of *H. pylori*-associated gastrointestinal disorders, but is not limited thereto.

The compound to be administered by the method of the invention is a compound which capable of inhibiting gastrin uptake by *H. pylori*. Particular compounds may be competitive inhibitors of gastrin uptake by *H. pylori* and/or antagonists of the human or *H. pylori* gastrin receptor.

In preferred embodiments, the compound to be administered is a peptide, preferably a synthetic analogue of gastrin or of a fragment of gastrin, preferably of G17. More preferred peptides the amino acid sequence: Trp-Met-Asp-PheNH_2, for example pentagastrin or cholecystokinin (CCK)-8.

In other embodiments of the method of the invention the compound to be administered may be a non-peptidic antagonist of the human or *H. pylori* gastrin receptor.

Still further, the invention relates to use of a compound which inhibits the growth-enhancing effect of gastrin on *H. pylori* in the preparation of pharmaceutical compositions for the treatment of *H. pylori*-associated disorders, particularly in the preparation of pharmaceutical compositions for the treatment of *H. pylori*-associated gastrointestinal disorders.

All of the compounds referred to above may be used in the preparation of pharmaceutical compositions according to the use of the invention.

The pharmaceutical compositions of the invention will generally contain salts, preferably in physiological concentration, such as PBS (phosphate-buffered saline), or sodium chloride (0.9% w/v), and a buffering agent, such as phosphate buffer in the above PBS. The preparation of pharmaceutical compositions is well known in the art, see e.g., US Patents Nos. 5,736,519, 5,733,877, 5,554,378, 5,439,688, 5,418,219, 5,354,900, 5,298,246, 5,164,372, 4,900,549, 4,755,383, 4,639,435, 4,457,917 and 4,064,236. The active ingredient of the pharmaceutical compositions of the present invention, for example a peptide, or pharmacologically acceptable salts thereof, is preferably mixed with an excipient, carrier, diluent, and optionally, a preservative or the like pharmacologically acceptable vehicles as known in the art, see e.g., the above US patents. Examples of excipients include, glucose, mannitol, inositol, sucrose, lactose, fructose, starch, corn starch, microcrystalline cellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, polyvinylpyrrolidone and the like. Optionally, a thickener may be added, such as a natural gum, a cellulose derivative, an acrylic or vinyl polymer, or the like.

The pharmaceutical composition is provided in solid, liquid or semi-solid form. A solid preparation may be prepared by blending the above components to provide a powdery composition. Alternatively, the pharmaceutical composition is provided as lyophilized preparation. The liquid preparation is provided preferably as aqueous solution, aqueous suspension, oil suspension or microcapsule composition. A semi-solid composition is provided preferably as hydrous or oily gel or ointment. About 0.001 to 60 w/v%, preferably about 0.05 to 25w/v % of the active agent is provided in the composition.

A solid composition may be prepared by mixing an excipient with a solution of the active agent comprised in the composition of the invention, gradually adding a small quantity of water, and kneading the mixture. After drying, preferably *in vacuo*, the mixture is pulverized. A liquid composition may be prepared by dissolving, suspending or emulsifying the active compound in water, a buffer solution or the like. An oil suspension may be prepared by suspending or emulsifying the active compound in an oleaginous base, such as sesame oil, olive oil, corn oil, soybean oil, cottonseed oil, peanut oil, lanolin, petroleum jelly, paraffin, Isopar, silicone oil, fatty acids of 6 to 30 carbon atoms or the corresponding glycerol or alcohol esters. Buffers include Sorensen buffer [Ergeb Physiol, (1912) **12**:393], Clark-Lubs buffer [J Bact (1917) 2(1):109 and 191]. Macllvaine buffer [J Biol Chem (1921) 49:183], Michaelis buffer (Die Wasserstoffinonenkonzentration, p. 186, 1914), and Kolthoff buffer [Biochem Z, (1926) **179**:410].

A composition may be prepared as a hydrous gel, e.g. for transnasal administration. A hydrous gel base is dissolved or dispersed in aqueous solution containing a buffer, and the said active agent, and the solution warmed or cooled to give a stable gel.

Preferably, the composition of the invention is administered through intravenous, intramuscular or subcutaneous administration. Oral administration is expected to be less effective, particularly where the active compound is a peptide, because the peptide may be digested before being taken up. Of course, this consideration may apply less to an active peptide invention which is modified as described above, e.g., by being cyclic peptide, by containing non-naturally occurring amino acids, such as D-amino acids, or other modification which enhance the resistance of the peptide to biodegradation. Decomposition in the digestive tract may be lessened by use of certain compositions, for instance, by confining the active compounds comprised in the compositions of the invention in microcapsules such as liposomes. The pharmaceutical composition of the invention may also be administered to other mucous membranes. The pharmaceutical composition is then provided in the form of a suppository, nasal spray or sublingual tablet. The dosage of the peptide of the invention may depend upon the condition to be treated, the patient's age, body weight, and the route of administration, and will be determined by the attending physician. Doses of active agent ranging from 0.1 μg/kg to 100 mg/kg or higher, preferably from 0.5 μg/kg to 5 mg/kg, more preferably 0.1μg/kg to 1mg/kg, most preferably about 100μg/kg.

The uptake of an active agent comprised in the composition of the invention, e.g. an active peptide, may be facilitated by a number of methods. For instance, a non-toxic derivative of the cholera toxin B subunit, or of the structurally related subunit B of the heal-labile enterotoxin of enterotoxic *E. coli* may be added to the composition, see US Patent 5,554,378.

Alternatively, the pharmaceutical composition of the invention may comprise a biodegradable polymer selected from poly-1,4-butylene succinate, poly-2,3-butylene succinate, poly-1,4-butylene fumarate and poly-2,3-butylene

succinate, incorporating the active compound, as the pamoate, tannate, stearate or palmitate thereof. Such compositions are described e.g., in US Patent 5,439,688.

Additionally, a composition of the invention may be a fat emulsion. The fat emulsion may be prepared by adding to a fat or oil about 0.1-2.4 w/w of emulsifier such as a phospholipid, an emulsifying aid, a stabilizer, mixing mechanically, aided by heating and/or removing solvents, adding water and isotonic agent, and optionally, adjusting adding the pH agent, isotonic agent. The mixture is then homogenized. Preferably, such fat emulsions contain an electric charge adjusting agent, such as acidic phospholipids, fatty acids, bilic acids, and salts therof. Acidic phospholipids include phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, and phosphatidic acid. Bilic acids include deoxycholic acid, and taurocholic acid. The preparation of such pharmaceutical compositions is described in US 5,733,877.

The invention will now be described in more detail on hand of the following Examples, which are illustrative only and do not in any sense limit the scope of invention, which is defined by the appended claims.

Examples

Materials and Methods

Bacterial cultures and growth analysis

Bacteria were obtained from clinical isolates cultured as part of the routine work up for *H. pylori* detection. No bacteria were specifically obtained for the study. Growth curve analysis was performed using five different isolates. Endoscopic diagnosis included both peptic pathologies and normal appearing mucosa. Following endoscopy, biopsies were kept in balanced salt solution for no more than one hour. Subsequently, biopsy specimens were minced and plated on chocolate or Colombia agar plats. To generate

microaerophilic conditions, bacteria were grown in sealed jars, using anaerobic gas generating kits (Oxoid, BR-38) at 37^{0}C. Bacteria were re-plated under similar conditions every 2-4 days. For experimentation, bacteria were transferred to brain-heart infusion or Brucella broth liquid media (Gibco), supplemented with 10% calf serum (Biological Industries, Beit Haemek, Israel). Each sample was inoculated with 0.02 OD 600 corresponding to 1.6×10^3 CFU/100μL. Gastrin or control peptides were added at different concentrations as indicated.

H. pylori growth necessitates microaerophilic conditions. Repeated sampling from the same tube would result in disruption of such conditions. To overcome this problem, in each experiment, multiple samples in the number of time points assessed were used. Each sample contained identical bacterial numbers and gastrin concentrations. A different sample was assessed at each time point. Growth was assessed by OD readings.

All bacteria were obtained from clinical isolates. Growth kinetic experiments with *C. jejuni* were done in similar conditions as described above for *H. pylori*. Growth kinetic experiments with *E. coli*, were performed under conditions similar to the above, in an aerobic ambient.

Gastrin and control peptides

Gastrin 17, cholecystokinin (CCK)-8 (fragment 26-33), pentagastrin, somatostatin 14 and epidermal growth factor (EGF) were obtained from Sigma (St. Louis, MO). Lyophilized peptides were dissolved in a stock solution containing acetic acid and water in a ratio of 1:1 to a concentration of 1 mg/ml, as suggested by the manufacturer and diluted with sterile deionized water to the indicated concentrations.

Radioactive gastrin uptake

To assess labeled gastrin uptake, bacterial pellets were resuspended in 200µl Brucella broth containing 500 nmol/l ^{125}I-labeled gastrin (IncStar Pharmatrade). Incubation was at either 4^{0}C or 37^{0}C for 45 minutes. Bacteria were washed three times in PBS, resuspended and incubated for 30 minutes in Brucella broth containing proteinase K (Promega) at a concentration of 25ng/ml. Subsequently, bacteria were washed, resuspended in 100µl PBS, out of which 10µl were blotted on nitrocellulose filters and autoradiography performed for 7 days. Incorporated ^{125}I was quantitated using a gamma counter.

Cold inhibition assay

For the cold inhibition assay, bacterial pellets were resuspended in 200µl Brucella broth containing 500 nmol/l ^{125}I-labeled gastrin. Unlabeled gastrin in increasing concentrations (1250-5000 nmol/l) was added concurrently. Bacteria were incubated for 45 minutes at 37^{0}C, after which they were extensively washed with PBS. Incorporation of ^{125}I-labeled gastrin was determined using a gamma counter. As controls, bacteria were incubated with ^{125}I-labeled gastrin (500 nmol/l) in the presence of CCK-8, pentagastrin, somatostatin or EGF at the indicated concentrations.

Results

To assess the effect of G17 on *H. pylori* growth, bacteria were grown with increasing gastrin concentrations. Addition of gastrin to the growth media had a dose-dependent effect on the growth curve. Gastrin shortened the lag time, increased the growth rate at the logarithmic phase and increased the final cell density at the stationary phase (Fig. 1A). Fig. 1B shows the growth of 5

different clinical isolates in the presence of gastrin at the 0 and 48h time points. As shown, a similar effect of gastrin was noted for all five isolates.

To test the specificity of the gastrin effect, growth kinetic studies were done using a slow growing intestinal microaerophilic bacterium and a Gram-negative aerobic intestinal bacterium. As shown in Fig. 2A-B, gastrin had no effect on the growth of *Campylobacter jejuni*, or *Escherichia coli*. To test whether other peptides might also have a positive growth effect similar to gastrin on *H. pylori*, *H. pylori* was grown in the presence of somatostatin, that is produced in the gastric antrum, and EGF, a growth factor peptide found within the gastric lumen. In addition, since in humans the gastrin and CCKB receptors are homologous, the gastrin/CCKB receptor agonists CCK-8 and pentagastrin, which have structural homology to gastrin, were tested for their effect on *H. pylori* growth. As shown in Fig. 2 C-F, none of the peptides had an effect on the growth of *H. pylori*, indicating that the positive growth effect of gastrin was specific.

To learn whether the uptake of gastrin to *H. pylori* was specific and whether gastrin was internalized by *H. pylori* or bound to the outer surface, *H. pylori* and control bacteria were incubated with ^{125}I-labeled gastrin, at 4^0C, or 37^0C. Following this initial incubation, bacteria were co-incubated with proteinase K, to digest surface proteins. Labeled gastrin incorporation was assessed by autoradiography and CPM determination. As shown in Fig. 3, the uptake of gastrin by the bacteria was specific for *H. pylori*, since no labeling was noted with control bacteria. Further, only *H. pylori* incubated at 37^0C incorporated the ^{125}I-labeled gastrin. Such temperature-dependence suggests that an energy-dependent mechanism is responsible for the uptake of gastrin. Moreover, no reduction in radioactive counts was noted following co-incubation with proteinase K (before proteinase K incubation - 311 CPM,

following incubation with proteinase K - 385 CPM), suggesting that the gastrin entered the bacteria and thus, was protected from proteinase K digestion.

To test whether gastrin entered the bacteria by a non-specific mechanism or, by interaction with a specific binding site, uptake of labeled gastrin was competed, using unlabeled gastrin and control peptides. As shown in Fig. 4, the uptake of labeled gastrin by *H. pylori* could be inhibited with an excess of unlabeled gastrin. Similar to unlabeled gastrin, co-incubation of bacteria in the presence of CCK-8 and pentagastrin inhibited labeled gastrin uptake in a dose-dependent manner. In contrast, no inhibition of gastrin uptake was shown by EGF or somatostatin.

The cold-ligand competition and inhibition of gastrin uptake by peptides known to activate shared receptors in humans, the temperature profile of gastrin binding and the protection against proteinase K digestion, all suggest the internal localization of a putative receptor or channel with specific structural restriction.

Claims:

1. A pharmaceutical composition for the treatment and/or prevention of *H. pylori*-associated disorders comprising as active ingredient a therapeutically effective amount of a compound which inhibits the growth-enhancing effect of gastrin on *H. pylori*, optionally further comprising pharmaceutically acceptable carriers, adjuvants or diluents.

2. A pharmaceutical composition according to claim 1, for the treatment and/or prevention of *H. pylori*-associated gastrointestinal disorders.

3. A pharmaceutical composition according to claim 1 or claim 2, wherein said compound is capable of inhibiting gastrin uptake by *H. pylori*.

4. A pharmaceutical composition according to any one of claims 1 to 3, wherein said compound is a competitive inhibitor of gastrin uptake by *H. pylori*.

5. A pharmaceutical composition according to any one of claims 1 to 3, wherein said compound is an antagonist of the human or *H. pylori* gastrin receptor.

6. A pharmaceutical composition according to any one of claims 1 to 5, wherein said compound is a peptide.

7. A pharmaceutical composition according to claim 6, wherein said peptide is a synthetic analogue of gastrin or of a fragment of gastrin, preferably of G17.

8. A pharmaceutical compound according to claim 7, wherein said peptide comprises the amino acid sequence: Trp-Met-Asp-PheNH_2.

9. A pharmaceutical composition according to claim 8, wherein said peptide is pentagastrin or cholecystokinin (CCK)-8.

10. A pharmaceutical composition according to claim 5, wherein said compound is a non-peptidic antagonist of the human or *H. pylori* gastrin receptor.

11. A pharmaceutical composition according to any one of claims 1 to 10, for the treatment and/or prevention of *H. pylori*-associated gastric and/or duodenal peptic diseases.

12. A method for the treatment and/or prevention of *H. pylori*-associated disorders in a patient in need of such treatment, comprising administering to said patient a therapeutically effective amount of a compound which inhibits the growth-enhancing effect of gastrin on *H. pylori*. or a therapeutically effective amount of a composition according to any one of claims 1 to 11.

13. A method according to claim 12, for the treatment and/or prevention of *H. pylori*-associated gastrointestinal disorders.

14. A method according to claim 13 or 14, wherein said compound is capable of inhibiting gastrin uptake by *H. pylori*.

15. A method according to claim 14, wherein said compound is a competitive inhibitor of gastrin uptake by *H. pylori*.

16. A method according to claim 12 or 13, wherein said compound is an antagonist of the human or *H. pylori* gastrin receptor.

17. A method according to any one of claims 12 to 16, wherein said compound is a peptide.

18. A method according to claim 17, wherein said peptide is a synthetic analogue of gastrin or of a fragment of gastrin.

19. A method according to claim 18, wherein said peptide is a synthetic analogue of G17.

20. A method according to claim 19, wherein said peptide comprises the amino acid sequence: Trp-Met-Asp-PheNH_2.

21. A method according to claim 20, wherein said peptide is pentagastrin or cholecystokinin (CCK)-8.

22. A method according to any one of claims 12 to 16, wherein said compound is a non-peptidic antagonist of the human or *H. pylori* gastrin receptor.

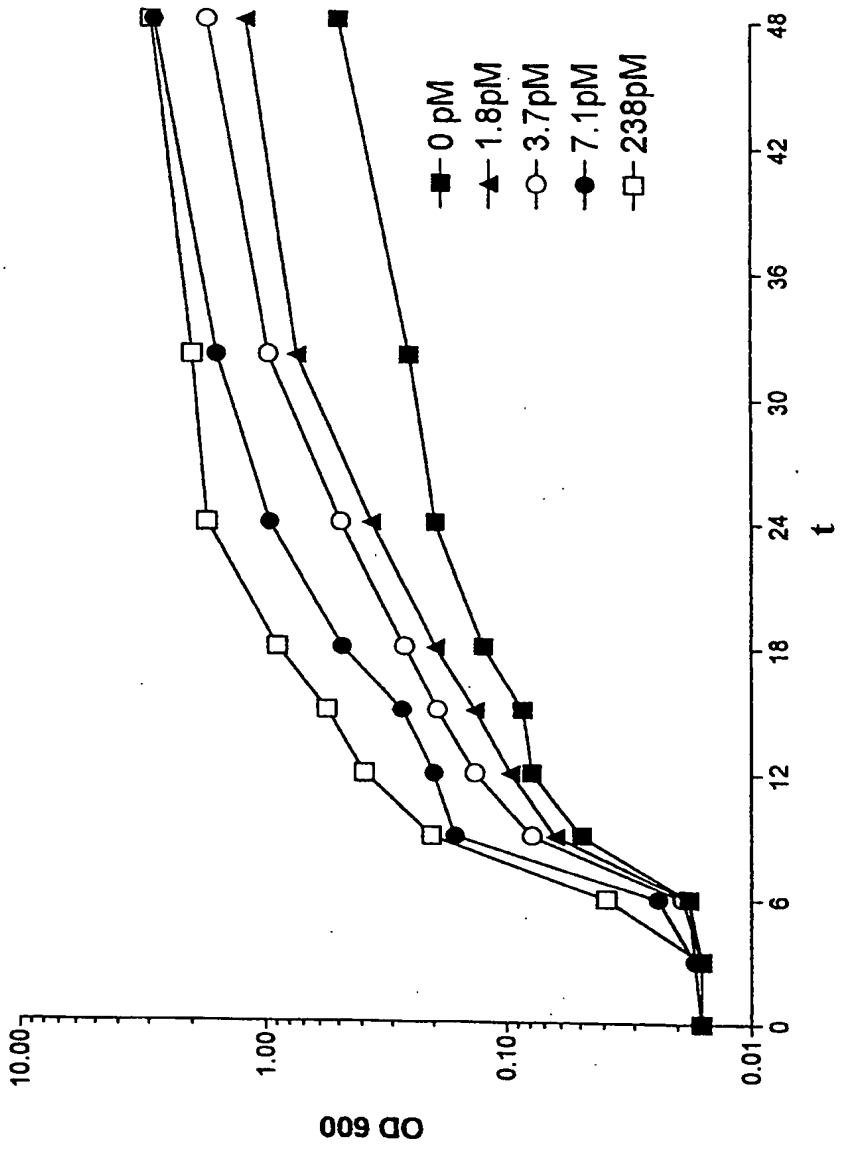
23. Use of a compound which inhibits the growth-enhancing effect of gastrin on *H. pylori* in the preparation of a pharmaceutical composition for the treatment of *H. pylori*-associated disorders.

24. Use according to claim 23, in the preparation of a pharmaceutical composition for the treatment of *H. pylori*-associated gastrointestinal disorders.

25. Use according to claim 30, wherein said compound is a synthetic analogue of G17, preferably comprising the amino acid sequence: Trp-Met-Asp-PheNH_2.

26. Use according to claim 30, wherein said compound is a non-peptidic antagonist of the human or *H. pylori* gastrin receptor.

Figure 1A


OD 600
10.00
1.00
0.10
0.01
0
6
12
18
24
30
36
42
48
t
0 pM
1.8pM
3.7pM
7.1pM
238pM

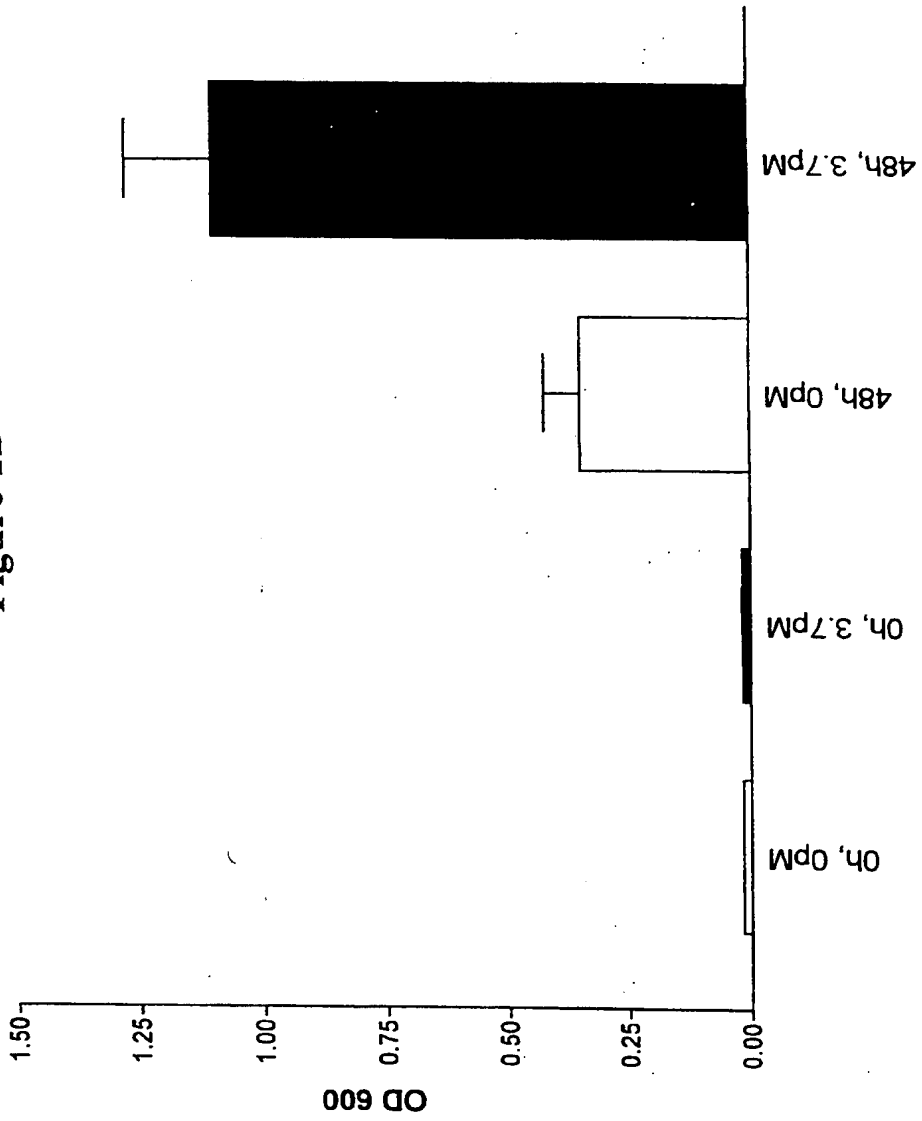

0h, 0pM
0h, 3.7pM
48h, 0pM
48h, 3.7pM
0.00
0.25
0.50
0.75
1.00
1.25
1.50
OD 600

Figure 1B

Figure 2A

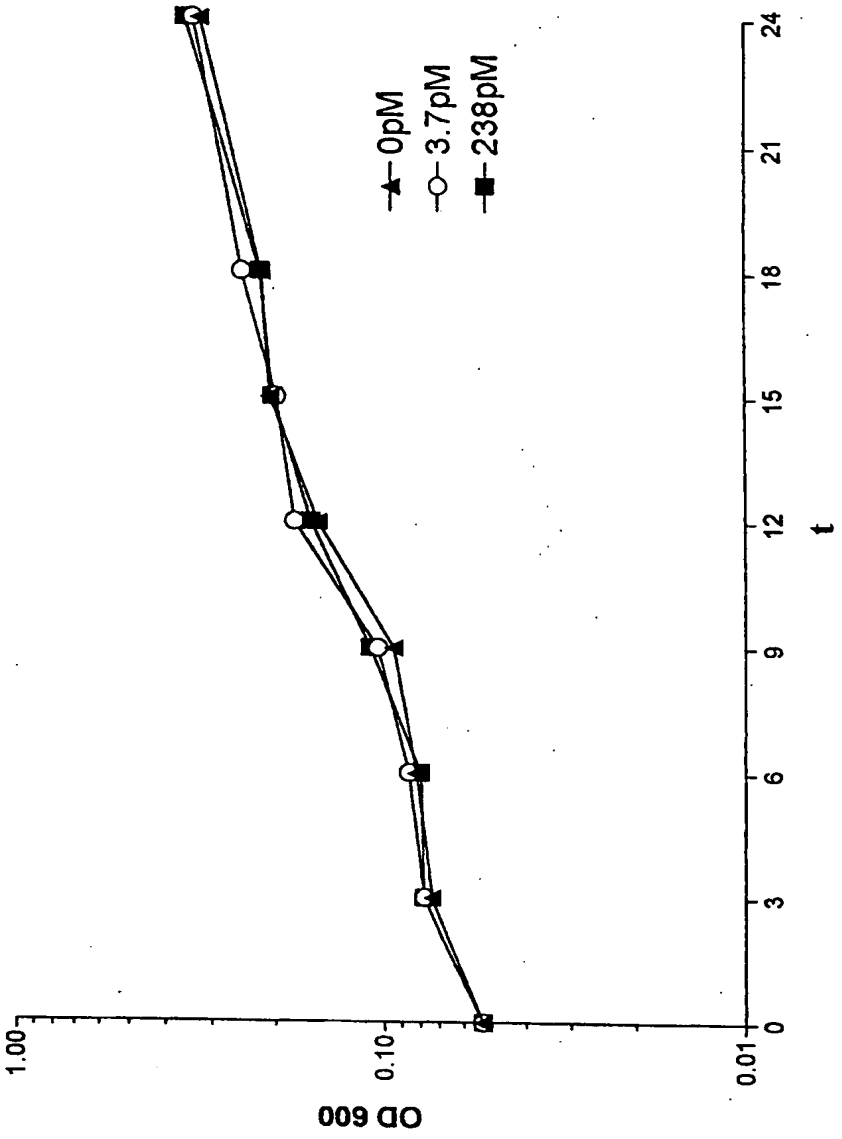
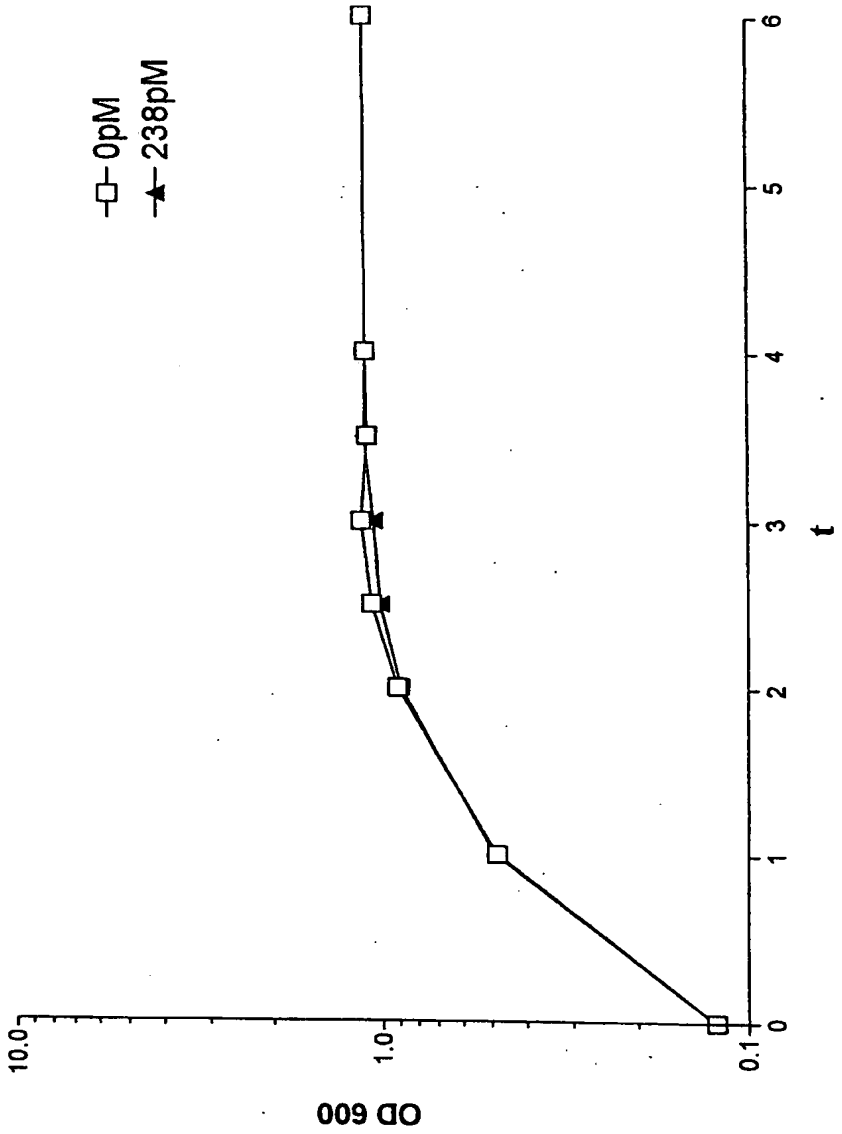


Figure 2B



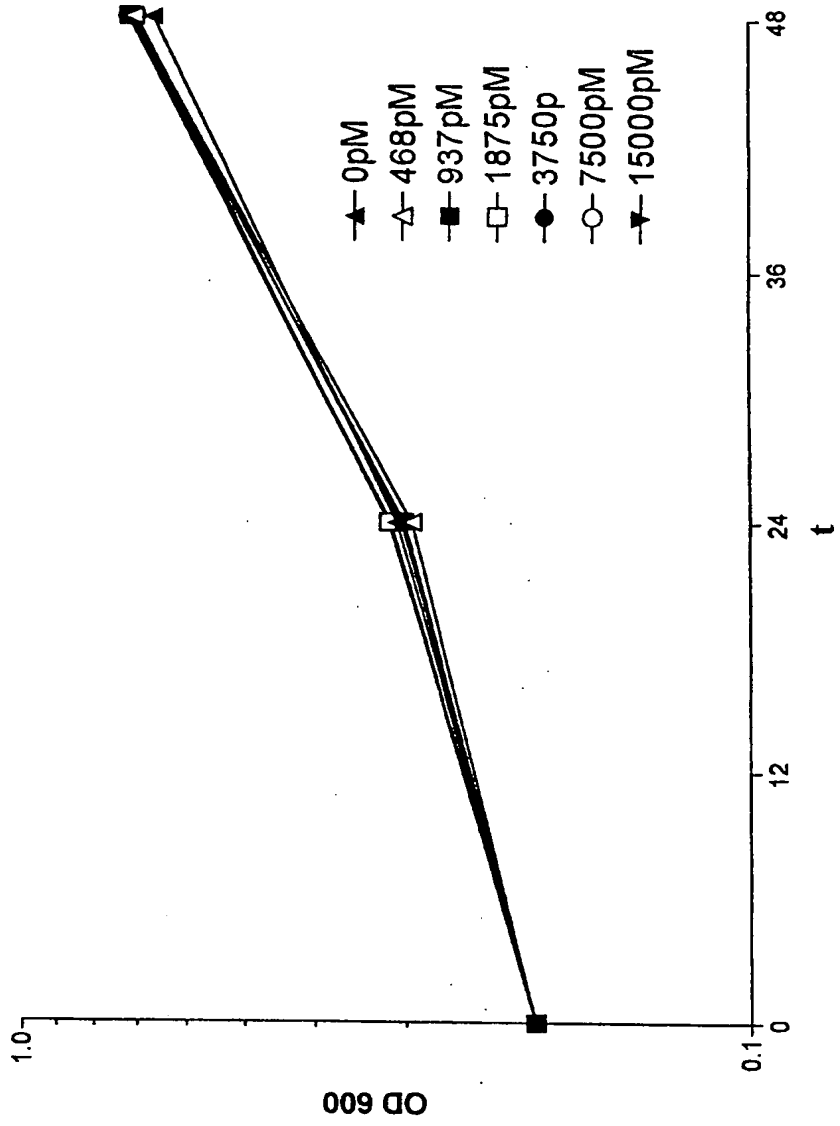
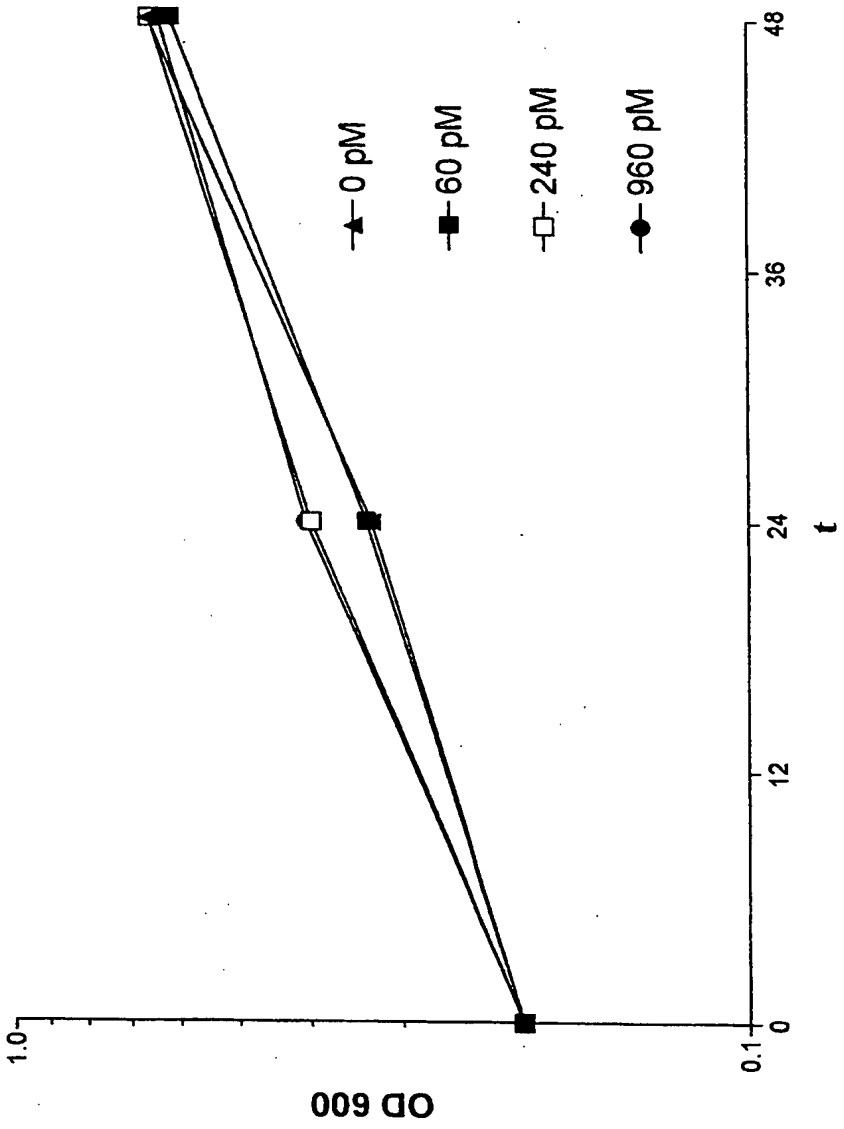

OD 600
1.0
0.1
0
12
24
36
48
t
0pM
468pM
937pM
1875pM
3750p
7500pM
15000pM

Figure 2C

Figure 2D



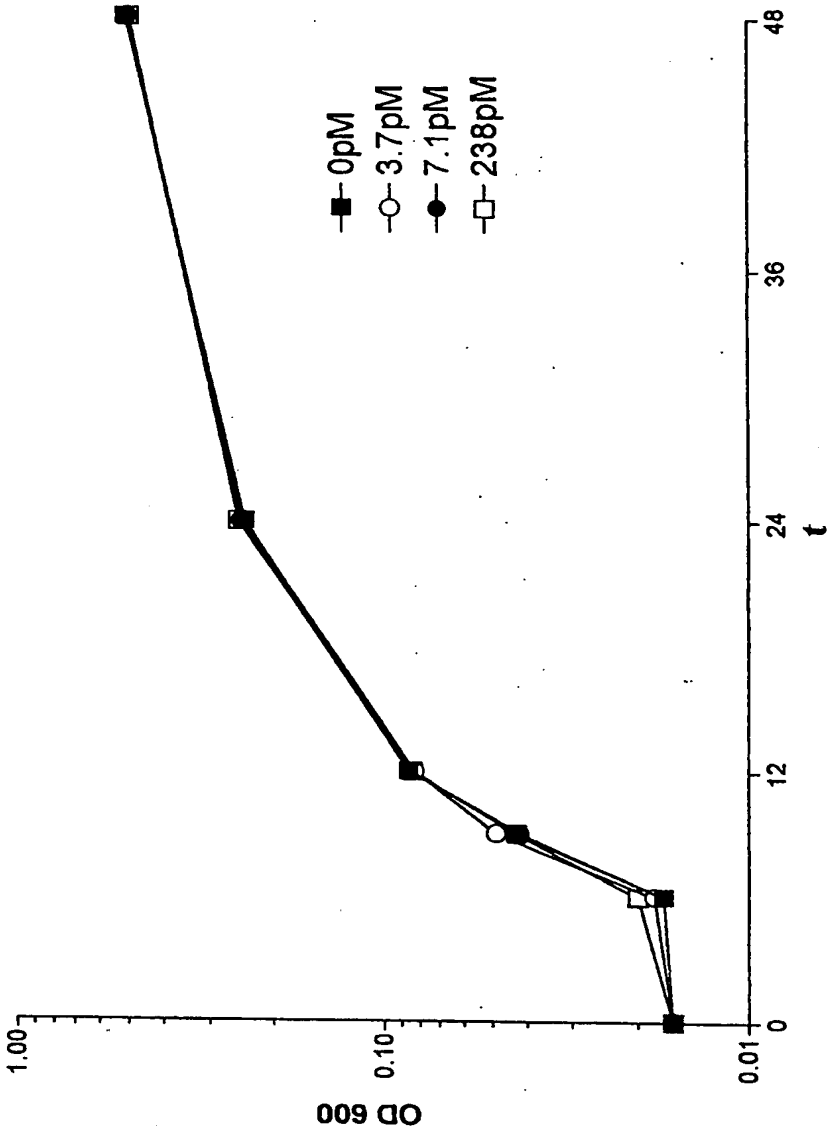


Figure 2E

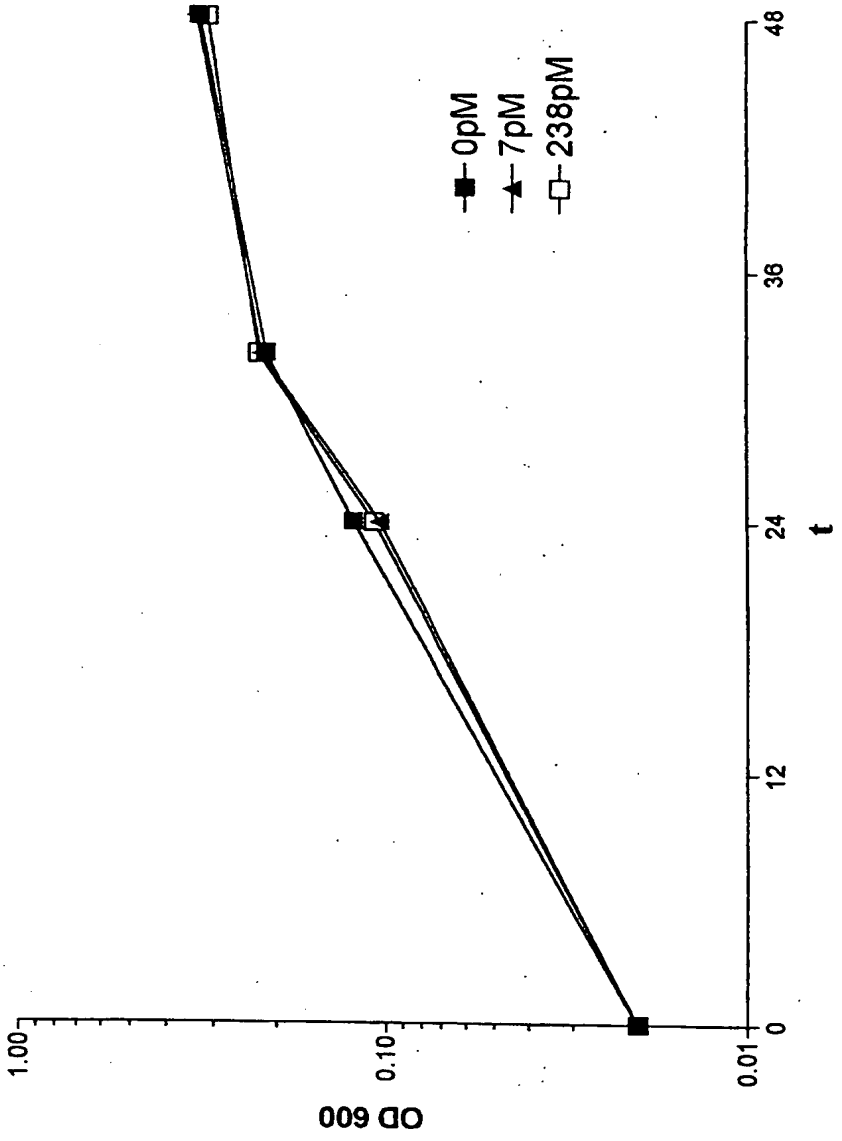
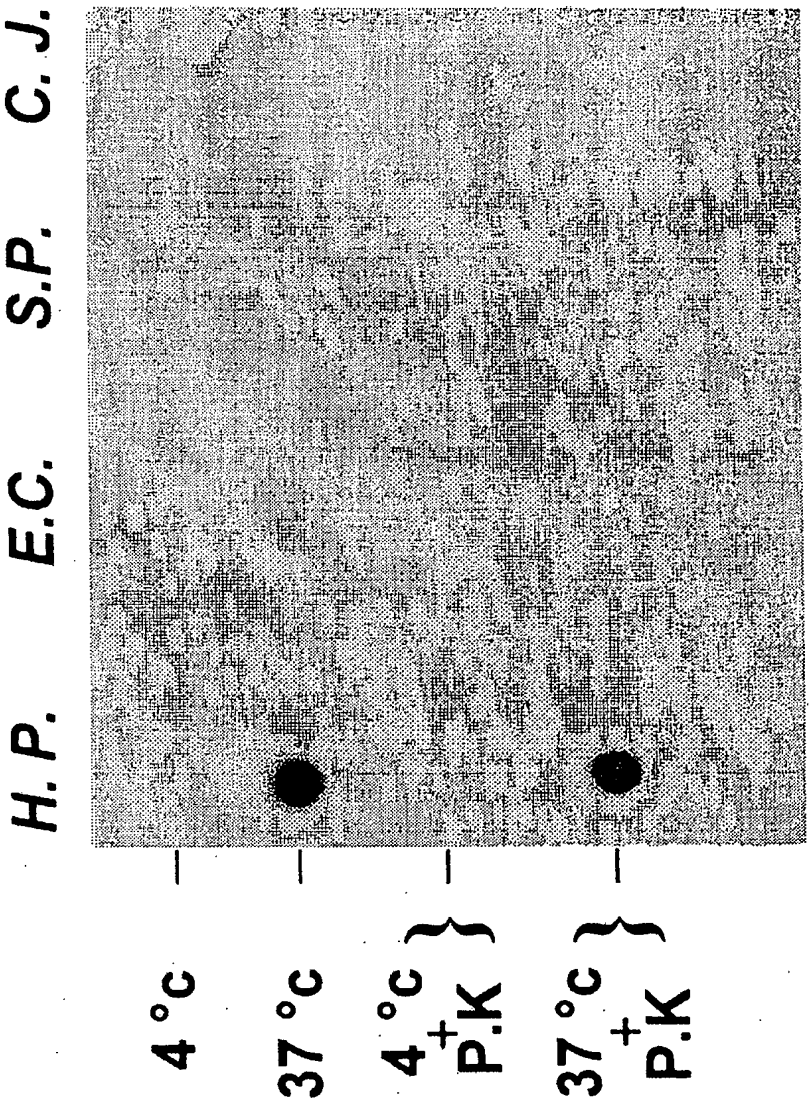


Figure 2F

Figure 3



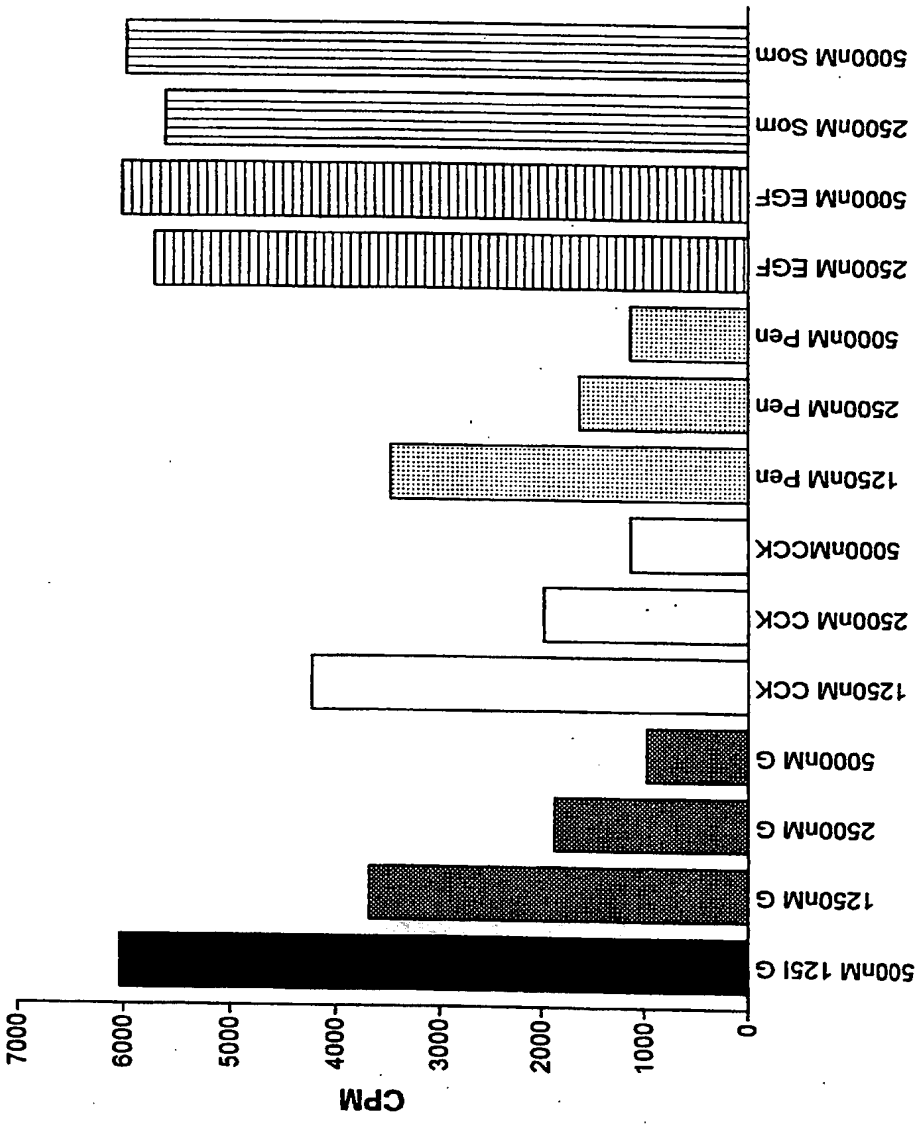

CPM
7000
6000
5000
4000
3000
2000
1000
0
500nM 125I G
125nM G
250nM G
500nM G
125nM CCK
250nM CCK
500nMCCK
125nM Pen
250nM Pen
500nM Pen
250nM EGF
500nM EGF
250nM Som
500nM Som

Figure 4

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 38/22, 38/07

A3

(11) International Publication Number: **WO 99/65513**

(43) International Publication Date: 23 December 1999 (23.12.99)

(21) International Application Number: PCT/IL99/00335

(22) International Filing Date: 17 June 1999 (17.06.99)

(30) Priority Data:

60/089,770	18 June 1998 (18.06.98)	US
60/147,195	20 May 1999 (20.05.99)	US

(71)(72) Applicants and Inventors: CHOWERS, Michal, Y. [IL/IL]; P.O. Box 77, 44925 Tzufit (IL). CHOWERS, Yehuda [IL/IL]; P.O. Box 77, 44925 Tzufit (IL).

(74) Agents: LUZZATTO, Kfir et al.; Luzzatto & Luzzatto, P.O. Box 5352, 84152 Beer-Sheva (IL).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report: 2 March 2000 (02.03.00)

(54) Title: PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF *HELICOBACTER PYLORI*-ASSOCIATED DISORDERS

(57) Abstract

The invention relates to pharmaceutical compositions and methods for treating and/or preventing *Helicobacter pylori*-associated disorders, particularly disorders of the gastrointestinal tract. The pharmaceutical compositions comprise as active ingredient a therapeutically effective amount of a compound which inhibits the growth-enhancing effect of gastrin on *H. pylori*. The active ingredient may specifically be a compound which is capable of inhibiting gastrin uptake by *H. pylori*, and/or which is an antagonist of the human or *H. pylori* gastrin receptor.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

rnational Application No
PCT/IL 99/00335

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/22 A61K38/07

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE WPI Section Ch, Week 199738 Derwent Publications Ltd., London, GB; Class B03, AN 1997-410809 XP002126333 & JP 09 183764 A (MORISHITA ROUSSEL KK), 15 July 1997 (1997-07-15) abstract --- -/--	1-26

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 December 1999	12/01/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ryckebosch, A

2

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 99/00335

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,A	K. YAMASHITA ET AL.: "INHIBITORY EFFECT OF SOMATOSTATIN ON HELICOBACTER PYLORI PROLIFERATION IN VITRO." GASTROENTEROLOGY, vol. 115, no. 5, November 1998 (1998-11), pages 1123-1130, XP000857858 NEW YORK, US cited in the application page 1127, left-hand column, line 17 - line 21; figure 2 -----	1-26

2

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 99/00335

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 12-22 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

rnational Application No
PCT/IL 99/00335

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 9183764 A	15-07-1997	NONE	